Soil aggregate stability as affected by fertilization type under semiarid no-tillage conditions

Daniel Plaza-Bonilla,* Jorge Álvaro-Fuentes and Carlos Cantero-Martínez

D. Plaza-Bonilla and C. Cantero-Martínez, Crop and Forest Sciences Department, Unidad Asociada EEAD-CSIC, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain.

J. Álvaro-Fuentes, Departamento de Suelo y Agua. Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), POB 13034, 50080 Zaragoza, Spain.

*Corresponding author: D. Plaza-Bonilla. E-mail: daniel.plaza@pvcf.udl.cat

Phone number: (+34)973702522. Fax number: (+34)973238264

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Abstract

Agricultural management practices play an important role in soil organic carbon (SOC) protection within soil aggregates. However, there is a lack of information on the effects of nitrogen (N) fertilization on C protection within aggregates under no-tillage (NT) systems. The effects of organic fertilization (with pig slurry and poultry manure) and mineral N fertilization on soil aggregation and physical C protection dynamics under NT soils were investigated. Two experiments were established in a semiarid area of northeast Spain. In the organic fertilization experiment, treatment with pig slurry at two N rates (100 and 200 kg N ha^{-1}), poultry manure (100 kg N ha^{-1}) and a control (0 kg N ha^{-1}) treatment were compared. In the mineral fertilization experiment, increasing rates of N fertilizer (0, 40, 80, 120 and 160 kg N ha^{-1}) were compared. Water-stable macroaggregates (>0.250 mm) and their C concentration, the distribution of dry-sieved aggregates, total SOC and microbial biomass C (MBC) were quantified in the soil surface in two cropping seasons. Organic fertilizers slightly increased the proportion of water-stable macroaggregates but caused no differences in MBC, SOC or water-stable macroaggregate C concentration. In the mineral N fertilization experiment, similar water-stable macroaggregate, water-stable macroaggregate C and SOC concentrations were observed among N fertilizer doses. Overall differences in water-stable macroaggregates between sampling dates were greater than differences between fertilization treatments. Our study demonstrates that, in the short-term, the use of organic or mineral N fertilizers hardly improves the stability of the macroaggregates and their C protective capacity when NT is performed. This finding could be related to the

limitations imposed by water in the Mediterranean areas and the buffering effect of long-term NT adoption on soil aggregate stability and C protection.

Abbreviations

MBC, microbial biomass carbon; MWD, mean weight diameter; NT, no-tillage; SOC, soil organic carbon; SWC, soil water content

Introduction

In Mediterranean agroecosystems, NT adoption increases crop water availability, resulting in greater biomass production (Cantero-Martínez et al., 2003; Cantero-Martinez et al., 2007). Moreover, in these systems it has been demonstrated that the use and maintenance of NT over time improves soil aggregation and C protection (Martin Lammerding et al., 2011; Plaza-Bonilla et al., 2010; Plaza-Bonilla et al., 2013). However, there is a lack of information about the effects of fertilization on soil C protection within aggregates when NT is performed. Soil structure is a key factor for soil maintenance and for the physical and biological processes that it involves. Soil structure mediates a number of soil properties such as crop nutrient availability, crop residue decomposition, soil erosion and, crop productivity (Bronick and Lal, 2005). In Mediterranean dryland areas there is a severe risk of soil erosion, so the maintenance of a suitable soil structure is essential in order to maintain soil quality (Álvaro-Fuentes et al., 2008a). One main indicator of soil structure is aggregate stability (Six et al., 2000). Soil aggregation is controlled by the quantity and quality of SOC, soil biota, ionic bridging, clay and silt content, and the presence of carbonates and gypsum (Amézketa, 1999).

SOC has a predominant effect on soil structure because its quantity and quality can be modified by agricultural management practices (Abiven et al., 2009; Whalen and Chang, 2002). Soil aggregation is a dynamic process, so it is expected to vary temporally (Chan et al., 1994). Temporal aggregate dynamics is influenced by climatic conditions, agricultural management practices (e.g., tillage, N fertilization and cropping system), crop growth and the decomposition kinetics of the organic residues applied to the soil (Álvaro-Fuentes et al., 2008b). Several studies have reported an improvement in soil physical properties after the long-term application of organic fertilizers (Abiven et al., 2009; Aoyama et al., 1999b; Celik et al., 2004; Wortmann and Shapiro, 2008) because of the increase in SOC content (Aoyama et al., 1999a; Paustian et al., 1992; Triberti et al., 2008). However, other studies have reported that manure application could reduce aggregate size, mainly due to the dispersive agents such as monovalent cations in the manure (Whalen and Chang, 2002). The increase in SOC caused by the application of organic fertilizers is a direct result of the manure composition and an indirect result of the increased crop growth and crop residue in response to the nutrient supply (Whalen and Chang, 2002). Aoyama et al. (1999a) proposed that organic fertilizers increase the pool of particulate organic matter, promoting the formation of soil macroaggregates in the short-term, whereas in the long-term the organic residues could be transformed to mineral-associated C, contributing to aggregate stabilization. Moreover, the application of organic residues to the soil can increase the stability of soil aggregates because of the physical and chemical action of the molecules contained in the organic products and/or the increase in the hydrophobicity of soil aggregates (Abiven et al., 2009). Some authors have reported an increase in SOC when synthetic fertilizers are applied to the soil (Álvaro-Fuentes et al., 2012; Halvorson et al., 1999). Therefore, an increase in soil structural stability following the application of mineral N could be expected (Sainju et al., 2003). In contrast, greater SOC mineralization was observed when N fertilizers were used (Khan et al., 2007), reducing aggregate stability (Le Guillou et al., 2011). Therefore, it is unclear how mineral fertilizers can affect both soil aggregate stability and the physical protection of SOC.

Animal waste from a large livestock industry is a common crop fertilizer source used in Mediterranean Spain, (http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database, European Commission. 2012. Eurostat, accessed 1 Nov. 2012). Organic fertilizers include a broad range of organic materials with different characteristics and therefore different decomposition kinetics (Thuries et al., 2002). For example, because of its high ammonia content, pig slurry tends to show a similar behavior to mineral fertilizers in soils (Sánchez-Martin et al., 2010), whereas N mineralization in solid organic fertilizers such as poultry manure tends to be more time-persistent (Thuries et al., 2002). In Mediterranean dryland livestock production is typically combined with crop production, resulting in a variable availability of organic materials from diverse animal sources for crop fertilization. Investigating the effects of organic products on physical C protection within soil aggregates is therefore essential in those areas.

The main objective of our experiment was to study the effects of organic and mineral N fertilization on aggregate stability and carbon content under no-tillage. Our hypotheses were: (i) the use of organic fertilizers results in an increase in the proportion of water-stable macroaggregates; (ii) soil macroaggregate stability increases when more recalcitrant products (e.g., poultry manure) are used; and (iii) SOC can be expected to increase when mineral N fertilizers are applied, with a concomitant increase in water-stable aggregates.

Materials and Methods

Experimental sites

The study was performed in two NT experimental fields with different fertilization management established in 2007 and 2008 in northeast Spain. The site with organic fertilization (41º43’N, 1º27’E, Conill, Spain) and the site with mineral fertilization (41º42’N, 1º30’E, Sant Martí Sesgueioles, Spain) were 5 km apart. The main objective of both experimental fields was to study crop response to the application of different doses of mineral N and types and doses of the most common organic fertilizers in the area (i.e. pig slurry and poultry manure) in order to better advise the farmers in the area. Central Catalonia, where the two experimental fields were set up, is an area of intensive livestock production (swine and poultry) that generates substantial amounts of organic waste. Farmers therefore have a varied range of mineral and organic products with which to fertilize crops. N rates were chosen according to the usual fertilization management in the area and previous fertilization experiments performed in the area (Angás et al., 2006; Morell at el., 2011c).

The two sites have identical climatic conditions, with mean annual precipitation and potential evapotranspiration of 500 and 960 mm, respectively. Selected soil properties for both experiments are detailed in Table 1. Prior to the establishment of the experiments, both fields had been under NT for the previous 10 years with organic and mineral N fertilization at Conill and Sant Martí Sesgueioles, respectively. During that time, the typical pig slurry application rate in Conill was about 20 m^3 ha^{-1}, which represents about 100 kg N ha^{-1}.

Both experiments consisted of a randomized complete-block design with three replications. Plot size was 50 x 12 m in the organic N fertilization experiment and 50 x 6 m in the mineral N fertilization experiment.

Mineral N fertilization experiment

In October 2007, five mineral N doses were established: 0, 40, 80, 120 and 160 kg N ha^{-1}. One-third of the dose was applied before the seeding of the crop with ammonium sulphate (26% N). The other two-thirds were applied as top dressing at the tillering stage of the crop with ammonium nitrate (33% N). The cropping system consisted of an NT barley (*Hordeum vulgare L.)* monocropping. Planting was performed in October with a disc direct-drilling machine after the application of a total herbicide (1.5 L 36% glyphosate per hectare). Potassium fertilization was applied according to the results of soil analyses with KCl, as in the organic fertilization experiment. The crop was harvested by the end of June with a commercial medium-sized harvester. The straw residue was chopped and spread over the soil.

Organic fertilization experiment

In October 2008, four organic treatments were established: a control treatment without application, two treatments with two rates of pig slurry, 100 and 200 kg N ha^{-1} (PS100 and PS200, respectively) and one treatment with the application of 100 kg N ha^{-1} of poultry manure (PM100). All the organic fertilizers were obtained from commercial farms near the experiment and their main characteristics are presented in Table 2. PS100 and PM100 were applied before the seeding of the crop, whereas PS200 was split into half the dose before seeding and the other half at crop tillering, as is common in the area. Fertilizers were surface-applied using commercial machinery previously calibrated to apply the precise dose. Pig slurry was conventionally surface-spread via a vacuum tanker fitted with a splashplate. Dry poultry manure was applied with a rear-discharge, box-type spreader equipped with beaters that broadcast the manure over a width of 12 m. Both types of machinery are commonly used in the area by farmers.

The cropping system consisted in an NT rotation of wheat (*Triticum aestivum L.*) and barley. Planting was performed in October with a direct-drilling disk machine to a depth of 3 cm, after the application of a non-selective herbicide (2 L 36% glyphosate per hectare). No mineral N was applied during the experiment, but in order to meet the crop's potassium requirement, KCl was applied before seeding according to the results of soil analyses. The crop was harvested by the end of June with a commercial medium-sized harvester. The straw residue was chopped and spread over the soil.

Soil sampling and analyses

During the 2009-2010 and the 2010-2011 cropping seasons, soil surface (0–5 cm) was sampled on seven different dates corresponding to different crop developmental stages: tillering and post-application of the top-dressing fertilization on 19 March 2010; flowering on 12 May 2010; post-harvest on 19 July 2010; post-summer fallow and post-application of pre-seeding fertilization on 5 November 2010; tillering and post-application of top-dressing fertilization on 29 March 2011; flowering on 3 May 2011; and post-harvest on 25 July 2011. In each plot, two composite samples were obtained from two representative areas 10 m apart. Undisturbed soil samples were collected with a flat spade. Each soil sample was stored in a crush-resistant airtight container. From each composite sample, SWC was measured gravimetrically by drying soil subsamples at 105ºC until constant weight. Once in the laboratory, the undisturbed samples were passed gently through an 8-mm sieve and air-dried at room temperature. For each sample, water-stable macroaggregate (>0.250 mm) separation and dry soil aggregate distribution were performed. Water-stable macroaggregate (>0.250 mm) size separation was performed according to a modified wet sieving method adapted from Elliott (1986). Briefly, a 100-g air-dried soil sub-sample was placed on the top of a 0.250-mm sieve and submerged for 5 min in deionized water at room temperature. The sample was manually sieved 50 times for 2 min. The water-stable macroaggregates obtained were oven-dried at 50ºC for 24 h and weighed. Sand content of the macroaggregates (>0.050 mm) was determined by dispersing a 5-g sub-sample in sodium hexametaphosphate solution using a reciprocal shaker. Sand-corrected macroaggregates (g g^{-1} dry soil) were expressed as:

Sand-corrected macroaggregates =

= Water-stable macroaggregate weight/ [1- (sand $\text{proportion}_{\text{macroaggregates}}$)]

The dry aggregate size distribution was conducted by placing 100 g of air-dried soil sub-sample (8-mm-sieved) on an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain) with a series of three sieves (2, 0.250 and 0.050 mm) in order to obtain four aggregate fractions: (i) large macroaggregates (2–8 mm); (ii) small macroaggregates (0.250–2 mm); (iii) microaggregates (0.050–0.250 mm); and (iv) silt-plus clay-sized particles (<0.050 mm). A sieving time of 1 min and the lowest-power program of the machine were used. The mean weight diameter (MWD) (Youker and Mc Guiness, 1957) was used to express the dry soil aggregate distribution.

The organic C concentration of the bulk soil (SOC) and the water-stable macroaggregates (water-stable macroaggregate C) were determined using the wet oxidation method of Walkley-Black described by Nelson and Sommers (1996). The method was modified to increase the digestion of SOC. The modification consisted in boiling the sample and the extraction solution at 150ºC for 30 minutes (Mebius, 1960).

The microbial biomass C (MBC) in bulk soil (0–5 cm) was measured in the organic fertilization experiment during the last three sampling dates (March, May and July 2011) in order to elucidate possible effects on the stability of the macroaggregates. The analyses were performed according to the chloroform-fumigation and direct extraction method of Vance et al. (1987). The extracts were analyzed for organic C using a Shimadzu TOC-VCSH analyzer. The MBC extraction coefficient applied was 0.38 (Sparling and Zhu, 1993; Vance et al., 1987).

The data were analyzed using the SAS statistical software (SAS institute, 1990). To compare the effects of fertilizer treatments and sampling date, a repeated measures analysis of variance was performed for each site. When significant, differences among treatments were identified at the 0.05 probability level of significance using an LSD test. In the mineral N fertilization experiment the different rates of N were analyzed as continuous variables. In order to determine the relationships between some variables, linear regression analyses were performed with Sigmaplot 11 (Systat Software, Inc., 2008).

Results

Rainfall events and mean soil and air temperature during the whole experimental period are shown in Fig. 1. Soil temperature was below zero (ºC) for two weeks prior to the first sampling event during the tillering stage of the crop (March 2010) and one month before the March 2011 sampling . Moreover, a snowfall occurred two weeks before the March 2010 sampling. Soil and air temperature increased during the subsequent months, reaching their maximum in the post-harvest sampling events (July 2010 and July 2011). Rainfalls of 14, 5 and 18 mm occurred the week before the March 2010, May 2010 and May 2011 sampling dates, respectively. On the July 2010, November 2010, March 2011 and July 2011 sampling dates no rainfalls occurred.

Mineral N fertilization effects on soil aggregation dynamics and C protection

In the mineral N fertilization experiment, water-stable macroaggregates ranged between 0.12 and 0.28 g g^{-1} dry soil (Fig. 2). No differences in soil water-stable macroaggregates were found between mineral N fertilization doses (Table 3). However, when sampling dates were compared, significant differences arose (Fig. 2). The lowest proportion of water-stable macroaggregates was found in March 2010 (tillering and post-application of top-dressing fertilization) and the highest proportion in July 2010 (post-harvest).
The MWD of the dry-sieved aggregates ranged between 2.90 and 3.79 mm (Fig. 2), with significant differences between N doses and sampling dates (Table 3 and Fig. 2). The MWD increased significantly with the increase in the dose of N applied (data not shown). In turn, the highest values of MWD were found on the first two sampling dates (March and May, 2010), while the lowest values were found in July 2010 and July 2011 (Fig. 2).

Over the whole experimental period, total SOC concentration ranged between 16.6 and 25.7 g kg^{-1} dry soil (data not shown). No effects of N doses or the interaction between N doses and sampling dates on SOC or macroaggregate C were observed (Table 3). However, significant differences between sampling dates were found in SOC and macroaggregate C (Table 3). Both SOC and macroaggregate C concentration were highest in July 2011 (Table 4).

Organic fertilization effects on soil aggregation dynamics and C protection

The proportion of water-stable macroaggregates ranged between 0.27 and 0.50 g g^{-1} dry soil for the control treatment (i.e. 0 kg N ha^{-1}) in March 2010 and for the PS100 treatment in July 2010, respectively (data not shown). Significant differences in water-stable macroaggregates were found between organic fertilization treatments. When organic fertilizers were applied (PM100, PS100 and PS200), a greater proportion of water-stable macroaggregates was observed than in the control treatment (Table 5). Also, significant differences were found between sampling dates (Table 3), with the highest values in July 2010 and March 2011 (Fig. 3). However, no significant interaction was found between organic fertilization treatments and sampling dates. Also, significant differences were found between N fertilization treatments and sampling dates in MWD (Table 3). The highest MWD was observed in the PS200 and PM100 treatments (Table 5), whereas the lowest was observed in the control treatment (Table 5). When sampling dates were compared, the highest MWD was observed during the first three sampling events (March, May and July 2010) (Fig. 3).

No significant differences were found between organic fertilization treatments, sampling dates or their interaction on SOC (Table 3). However, the sampling date significantly affected the macroaggregate C concentration. Water-stable macroaggregate

C ranged between 78.0 and 105.0 for the PS200 treatment in March 2011 and for the PM100 treatment in May 2010, respectively (data not shown). No differences in MBC were found between organic fertilization treatments (Table 6). However, in the PS100 treatment, differences between sampling dates were found, with greater MBC in March 2011 and May 2011 than in July 2011 (Table 6).

Discussion

The addition of organic fertilizers slightly increased the proportion of soil-surface water-stable macroaggregates and the MWD of the dry-sieved aggregates (Table 5). On the other hand, the application of increasing doses of mineral N fertilizer only affected the MWD of the dry-sieved aggregates (Table 3). This observation suggests that unstable aggregates are formed when N mineral fertilizers are applied. Consequently, in our experiment, N fertilization (both organic and mineral) had a minor effect on soil surface aggregation. As stated in the Materials and Methods section, soil management prior to the establishment of both experiments consisted of NT. In both experiments, the use of NT for more than 10 years could have resulted in the development of soil macroaggregates with high stability, in turn diminishing the possibility of any subsequent effect due to the application of fertilizers. Also, because both experiments have been conducted for only a few years, it could be hypothesized that effects of greater magnitude may occur after the long-term use of fertilizers. Under NT soil macroaggregates are more stable, resulting in greater protection of SOC (Álvaro-Fuentes et al., 2008b; Plaza-Bonilla et al., 2010). Aoyama et al. (1999b) concluded that manure application can provide a C-protective mechanism in annually tilled cropping systems similar to that provided by NT. In a soil management and N source experiment, Mikha and Rice (2004) observed greater water-stable large macroaggregates with the application of manure combined with NT adoption. However, they found no significant interaction between soil management and N source. In our organic fertilization experiment, the historical fertilization management prior to the establishment of the experimental field consisted of pig slurry. This organic addition for more than 10 years could have led to the high initial levels of surface SOC measured in the experiment (between 46.9 and 53.8 g kg^{-1} dry soil) (Table 4). The initial high SOC could have contributed to the low differences in water aggregate stability found between organic fertilization treatments. In the organic fertilization experiment, our results show a small significant linear relationship between the proportion of water-stable macroaggregates and the total SOC (data not shown). However, the R^2 values obtained are considerably lower than those reported by other authors (e.g., Chaney and Swift, 1984) indicating that macroaggregates could be C-saturated. Fortun et al. (1989) concluded that the impact of addition of organic products to the soil is greater in cases in which the initial concentration of soil C is low. Likewise, Stewart et al. (2008) demonstrated that soils that are near to C-saturation are less efficient than C-depleted ones when storing added C.

As stated above, no effects of mineral N fertilization on water-stable macroaggregates were found (Table 3). This lack of response has also been reported by other authors (e.g., Aoyama et al., 1999a; Celik et al., 2004; N'Dayegamiye, 2009). Moreover, the application of N fertilizer affected neither SOC concentration nor water-stable macroaggregate C concentration (Table 3). Other authors have reported a lack of response of SOC or macroaggregate C to N mineral fertilization (Aoyama et al., 1999b; Mikha and Rice, 2004). In rainfed Mediterranean agroecosystems there is limited response to N application, mainly because of the restricted soil water availability for crop growth (Cantero-Martínez et al., 1995; Cantero-Martínez et al., 2003). In these systems, N fertilization has little impact on C inputs returned to the soil (crop residues and roots) (Morell et al., 2011a, Morell et al., 2011c) and, as a result, its effects on SOC tend to appear in the long term (Alvaro-Fuentes et al., 2012; Morell et al., 2011b). Although differences in biomass production between N fertilization doses have been observed in the experiment (data not shown), the small number of years since its implementation could have influenced the absence of differences in SOC. Other authors have reported a negligible or slight soil C sequestration under rainfed Mediterranean conditions when using mineral fertilizers (López-Bellido et al., 2010; Triberti et al., 2008).

We hypothesized that the application of recalcitrant organic residues (e.g., poultry manure) would be followed by a more constant stability of macroaggregates when compared with more labile organic products such as pig slurry. It has been reported that the C composition of pig slurry is more labile than that of solid organic fertilizers (Gigliotti et al., 2002; Bol et al., 2003). Thus, fast mineralization of the most labile parts of the C applied with the pig slurry could increase the water stability of the aggregates. Contrary to our hypothesis, no significant interaction was found between the N treatment and the date of sampling, demonstrating the absence of different temporal responses in macroaggregate stability when organic residues of different composition are applied.

According to the repeated-measures analyses of variance performed, the effect of the sampling date on water-stable macroaggregates, MWD and macroaggregate C concentration was significant in both experiments because of the high seasonal variation in these variables (Table 3). SOC was only significantly affected by the sampling date in the mineral N fertilization experiment. However, as stated above, no significant interaction between sampling date and fertilization treatment was found for any of the variables measured. In both experiments, the lowest proportion of water-stable macroaggregates was observed on the first sampling date (March 2010) (Figs. 2 and 3). As stated in the Results section, two weeks before the first sampling date a snowfall accompanied by low temperatures occurred. It is known that rapid rewetting of the soil and freeze-thaw cycles cause swelling (Kemper et al., 1985; Dagesse, 2011) that can be followed by slaking and macroaggregate disruption (Denef et al., 2001). These factors could have reduced the stability of macroaggregates during that period. The great differences between sampling dates in water-stable macroaggregates in both experiments contrasts with the slight or null effect of the N treatments (in the organic and mineral N fertilization experiments, respectively) on that variable. Some authors have reported seasonal variations in aggregate stability greater than the differences between the treatments that they were comparing (Alderfer, 1950; Chan et al., 1994; Perfect et al., 1990). For example, a negative correlation between the SWC and the soil aggregate stability was reported by Angers (1992), Chan et al. (1994) and Yang and Wander (1998). However, unlike earlier studies, our experiment did not show a meaningful relation between macroaggregate stability and SWC. In the mineral N fertilization experiment, the greatest proportion of water-stable macroaggregates was found on the sampling date of July 2010. This fact contrasts with the SOC and water-stable macroaggregate C concentration on that sampling date, which showed their lowest values (Table 4). Perfect et al. (1990) studied the impact of soil moisture, roots and microbial biomass on temporal fluctuations in soil structural stability. They concluded that there was a dominant influence of soil water content on the stability of the soil surface. Water content at the time of sampling is clearly related to the forces of soil cohesion. Kemper and Rosenau (1984) found that soil cohesion decreased with an increase in soil water content. Also, Blanco-Moure et al. (2012) pointed out the deleterious effect of rains after long dry periods in the Mediterranean areas and demonstrated that slaking could be the dominant disaggregation process during these events.

In the Mediterranean cropping systems, the rainfall distribution pattern is bimodal, with the greatest peaks of rainfall in September-October and, to a lesser extent, in April-May (Austin et al., 1998). In these systems, during the spring months winter cereal crops are in the flowering stage of high water consumption. Thus, in spring 2010 and 2011, lower SWC in the spring months could contribute to the greater proportion of water-stable macroaggregates found during these periods. Moreover, in the flowering period, root biomass could be considered at its maximum (Lampurlanés et al., 2001; Morell et al., 2011a), also enhancing the stability of soil macroaggregates (Jastrow et al., 1998). Jastrow and Miller (1997) also pointed out the role of root exudates in the stabilization of soil aggregates during their decomposition.

In a similar Mediterranean area and using a similar experiment to ours, Álvaro-Fuentes et al. (2007) studied the effects of soil tillage and cropping system on aggregate dynamics. They also found lower aggregate stability values in winter than in summer. However, they concluded that MBC was the main factor affecting water stability of aggregates. In our study, the relationship found between MBC and water-stable macroaggregates was low (data not shown). Perfect et al. (1990) also concluded that microbial biomass played a secondary role in controlling water aggregate stability during time one.

Conclusions

The application of organic fertilizers to the soil increased the proportion of water-stable macroaggregates. However, poultry manure did not provide greater macroaggregate stability than pig slurry and, in general, the application of none of these products increased the protection of C within aggregates or the total SOC concentration of the bulk soil. The use of increasing doses of mineral N fertilizer did not increase the stability of macroaggregates. The seasonal changes in soil macroaggregates stability had more impact than fertilization treatments (organic and mineral N). Our study demonstrates that, in the short-term, the use of organic or mineral N fertilizers hardly improves the stability of the macroaggregates and their C-protective capacity when NT is performed. This fact could be related to the limitations imposed by water in the Mediterranean areas and the buffering effect of long-term NT adoption on soil aggregate stability and C protection.

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FIGURE CAPTIONS

Fig. 1 Soil temperature (dotted line), air temperature (continuous line) and rainfall events (columns) during the experimental period. Small vertical arrows indicate soil sampling events.

Fig. 2 Proportion of water-stable macroaggregates (>0.250 mm) and mean weight diameter of the dry-sieved aggregates **(**MWD) in soil surface (0–5 cm depth) of the mineral fertilization experiment as affected by sampling date. Values are the means of the five mineral N fertilizer rates compared (0, 40, 80, 120 and 160 kg N ha^{-1}). For each variable, different lowercase letters indicate significant differences between sampling dates at $P<0.05$. Vertical arrows indicate the application of fertilizers.

Fig. 3 Proportion of water-stable macroaggregates (>0.250 mm) and mean weight diameter of the dry-sieved aggregates **(**MWD) in soil surface (0–5 cm depth) of the organic fertilization experiment as affected by sampling date. Values are the means of the four organic fertilization treatments compared (a control treatment, pig slurry at 100 and 200 kg N ha^{-1} and poultry manure at 100 kg N ha^{-1}). For each variable, different lowercase letters indicate significant differences between sampling dates at $P<0.05$. Vertical arrows indicate the application of fertilizers.

Table 1 Site and general soil characteristics in the 0-30 cm soil depth of the two experimental sites.

Site and soil characteristics	Organic fertilization	Mineral fertilization
Year of establishment	2008	2007
Latitude	41º 43’ N	41º 42’ N
Longitude	1º 27’ E	1º 30’ E
Elevation (m)	748	625
pH (H_2O, 1,2.5)	7.8	7.9
$EC_{1,5}$ (dS/m)	0.39	0.30
$CaCO_3$ eq. (%)	32	24
Particle Size Distribution (%)		
Sand (2000–50 µm)	13.1	23.5
Silt (50–2 µm)	47.2	41.1
Clay (<2 µm)	39.7	35.4

Table 2 Composition of the pig slurry (PS) and the poultry manure (PM) applied in the organic fertilization experiment (values are expressed as g 100 g^{-1} of dry matter)

Organic fertilizers characteristics	PS				PM	
	Pre-seeding 2009/10	Tillering 2009/10	Pre-seeding 2010/11	Tillering 2010/11	Pre-seeding 2009	Pre-seeding 2010
Dry matter	4.3	4.3	3.6	3.1	55.6	58.1
C	-	42.4	-	-	-	39.1
N kjeldahl	3.2	3.0	3.5	4.4	6.1	3.9
Ammonium-N	6.8	9.2	10.2	10.0	0.8	1.5
P	2.5	2.2	2.2	3.0	1.4	1.5
K	3.8	5.4	5.6	5.4	3.2	2.9

Table 3 ANOVA *P*-values showing significant differences in the proportion of water-stable macroaggregates (>0.250 mm), the mean weight diameter of the dry-sieved aggregates (MWD), the soil organic carbon of the bulk soil (SOC) and the water-stable macroaggregates C as affected by organic and mineral N fertilization treatments, sampling dates and their interaction.

Variables	Source of variation	Organic fertilization	Mineral N fertilization
Water-stable macroaggregates	N Treatment	*	ns
	Date	***	***
	Date*N treatment	ns	ns
MWD	N Treatment	**	***
	Date	***	***
	Date*N treatment	ns	ns
SOC	N Treatment	ns	ns
	Date	ns	***
	Date*N treatment	ns	ns
Macroaggregate-C	N Treatment	ns	ns
	Date	**	***
	Date*N treatment	ns	ns

ns: non-significant; *P<0.05; **P<0.01; *** P<0.001

Table 4 Soil organic carbon concentration (SOC) and water-stable macroaggregate C in soil surface (0–5 cm depth) in the organic and mineral fertilization experiments as affected by sampling date.

Variables	Sampling date	Organic fertilization	Mineral fertilization
SOC ($g\ kg^{-1}$ dry soil)	March 2010	46.9 (7.9)†	20.4 (1.6) c‡
	May 2010	47.7 (5.6)	21.2 (1.4) bc
	July 2010	49.6 (3.4)	18.7 (3.1) d
	November 2010	50.7 (3.3)	21.5 (1.4) bc
	March 2011	53.8 (2.5)	22.1 (1.9) ab
	May 2011	48.4 (6.0)	22.6 (3.4) ab
	July 2011	49.2 (2.9)	23.1 (1.4) a
Water-stable macroaggregate-C ($g\ kg^{-1}$ dry soil)	March 2010	93.6 (13.3) abc	44.6 (3.8) c
	May 2010	100.4 (22.9) a	48.3 (3.4) b
	July 2010	86.9 (14.8) bcd	41.0 (2.8) d
	November 2010	85.3 (13.7) cd	45.1 (4.7) c
	March 2011	80.5 (8.9) d	44.3 (3.5) c
	May 2011	85.5 (8.8) bcd	44.0 (6.5) c
	July 2011	94.1 (12.6) ab	53.6 (4.8) a

†Values in parenthesis are the standard errors of the mean.

‡Within each experiment (i.e. organic and mineral fertilization) and variable, different letters indicate significant differences between sampling dates at $P<0.05$.

Table 5 Proportion of water-stable macroaggregates (>0.250 mm) and mean weight diameter of the dry-sieved aggregates (MWD) in soil surface (0–5 cm depth) as affected by organic fertilization treatments with PS100 and PS200 (pig slurry 100 and 200 kg N ha^{-1}, respectively) and PM100 (poultry manure 100 kg N ha^{-1}). Values are the means of the seven sampling events.

Treatment	Water-stable macroaggregates	MWD
	g g^{-1} dry soil	mm
Control 0 kg N ha^{-1}	0.39 b†	3.02 c
PM100	0.43 a	3.23 ab
PS100	0.43 a	3.15 bc
PS200	0.44 a	3.30 a

†Within the same variable, different lowercase letters indicate significant differences between treatments at *P*<0.05.

Table 6 Soil microbial biomass C (MBC) in soil surface (0–5 cm depth) as affected by organic fertilization with PS100 and PS200 (pig slurry 100 and 200 kg N ha^{-1}, respectively) and PM100 (poultry manure 100 kg N ha^{-1}) in March 2011, May 2011 and July 2011.

Variable	Sampling date	Control 0 kg N ha^{-1}	PM100	PS100	PS200
MBC (mg C kg^{-1})	March 2011	810.6 (93.3)†	1229.8 (533.0)	1032.2 (145.8) a‡	1135.9 (179.3)
	May 2011	899.9 (172.9)	959.7 (250.9)	1082.4 (297.3) a	1372.4 (38.3)
	July 2011	590.6 (114.6)	579.2 (116.9)	654.6 (135.3) b	975.7 (218.6)

†Values in parenthesis are the standard errors of the mean.

‡Within each fertilization treatment, different letters indicate significant differences between sampling dates at $P<0.05$.

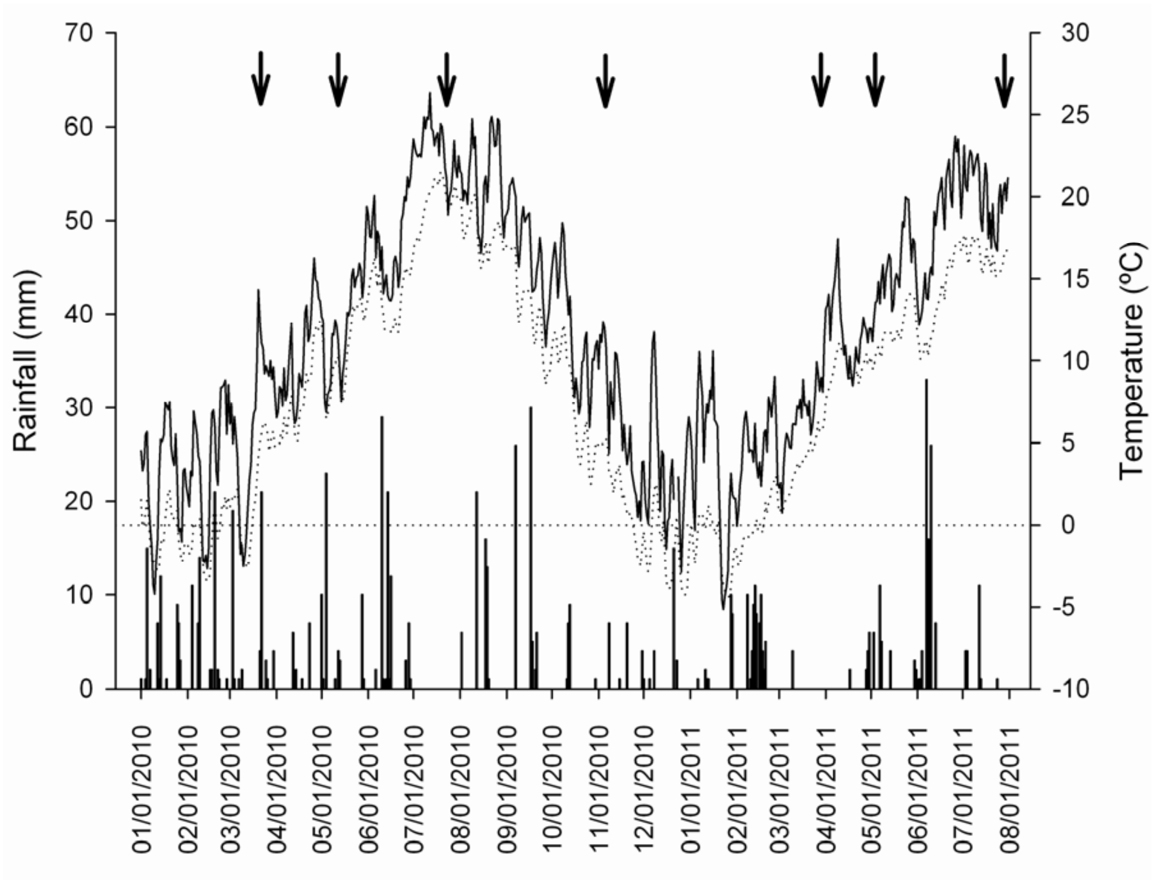

Fig. 1 Soil temperature (dotted line), air temperature (continuous line) and rainfall events (columns) during the experimental period. Small vertical arrows indicate soil sampling events.

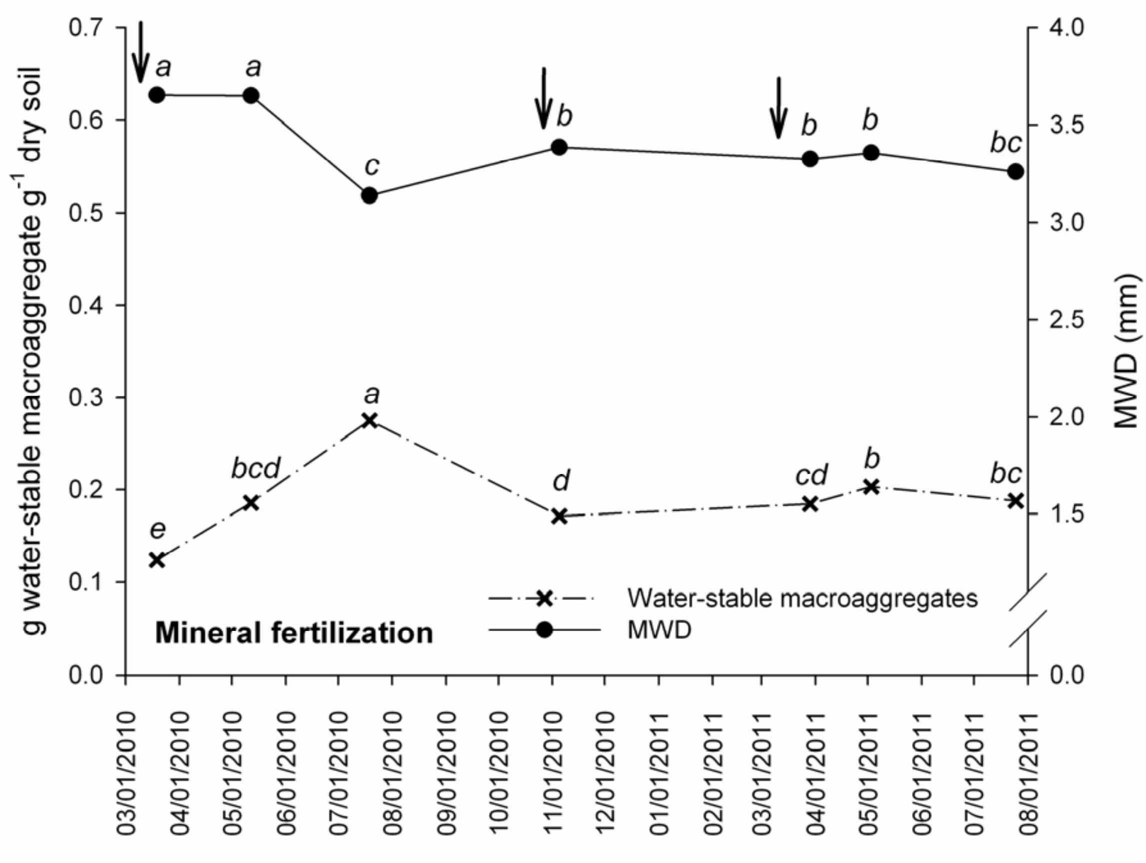

Fig. 2 Proportion of water-stable macroaggregates (> 0.250 mm) and mean weight diameter of the dry-sieved aggregates **(**MWD) in soil surface (0–5 cm depth) of the mineral fertilization experiment as affected by sampling date. Values are the means of the five mineral N fertilizer rates compared (0, 40, 80, 120 and 160 kg N ha^{-1}). For each variable, different lowercase letters indicate significant differences between sampling dates at *P*<0.05. Vertical arrows indicate the application of fertilizers.

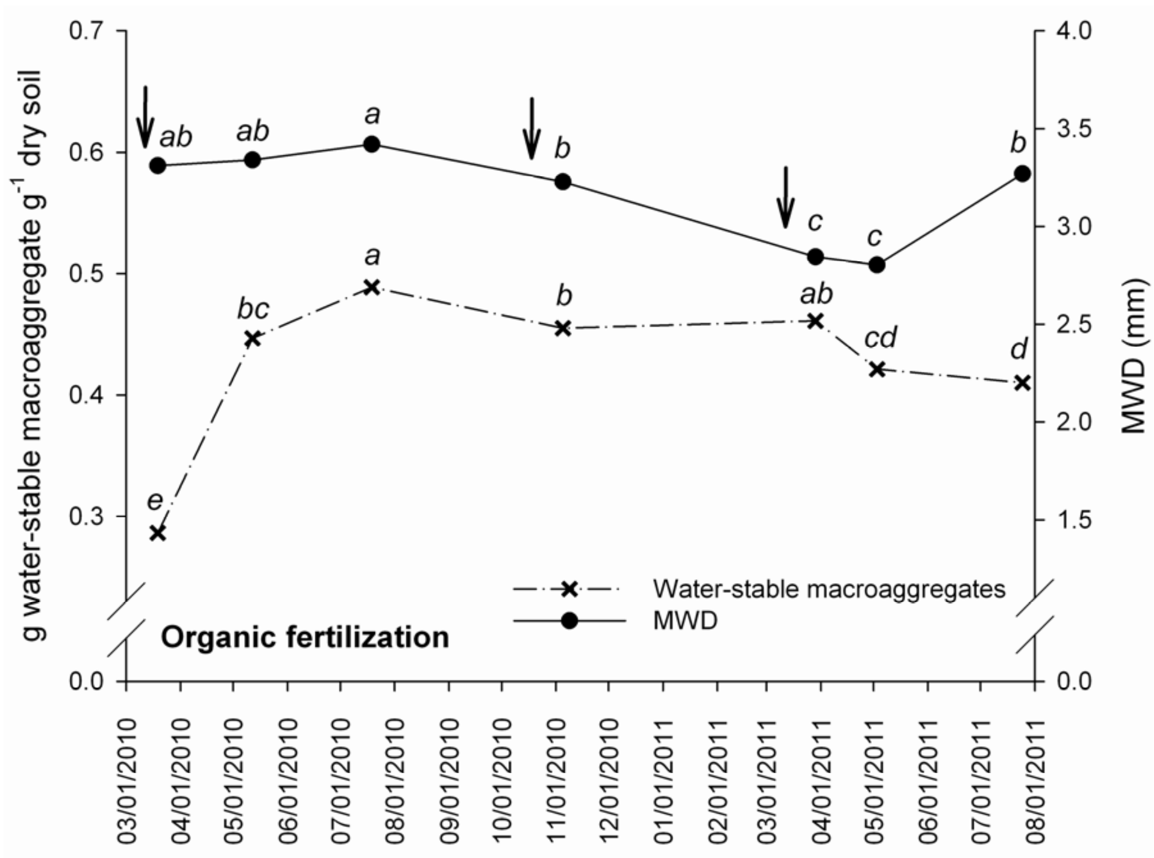

Fig. 3 Proportion of water-stable macroaggregates (>0.250 mm) and mean weight diameter of the dry-sieved aggregates **(**MWD) in soil surface (0–5 cm depth) of the organic fertilization experiment as affected by sampling date. Values are the means of the four organic fertilization treatments compared (a control treatment, pig slurry at 100 and 200 kg N ha^{-1} and poultry manure at 100 kg N ha^{-1}). For each variable, different lowercase letters indicate significant differences between sampling dates at P<0.05. Vertical arrows indicate the application of fertilizers.